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Letter

Why PB28 Could Be a Covid 2019 Game Changer?

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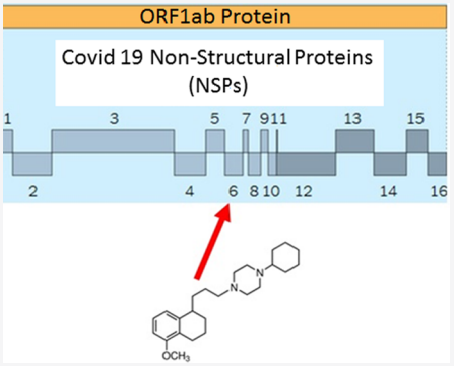
ABSTRACT: PB28, a cyclohexylpiperazine derivative, could be a potential strategy for Covid 19 because in a recent study it has been found more active than hydroxychloroquine without interaction with cardiac proteins. PB28 has been designed, developed, and biologically evaluated in the past decade in our research group. A possible mechanism to explain its surprising anti-COVID-19 activity is suggested..



KEYWORDS: *Cyclohexylpiperazine, PB28, NSP6, Covid 19, sigma receptors*

Recently, Gordon et al.[1] published a broad list of compounds that could be entertained for use in Covid-19 treatment waiting for a vaccine. The low cost strategy is the drug repurposing and, among the classes of compounds that the researchers have studied, potent and selective sigma-1 and sigma-2 receptor ligands have been enclosed. In that paper, sigma ligands and other off-label drugs with different drug status (approved, clinical, and preclinical) have been investigated, such as haloperidol, olanzapine, pimozide, clemastine, zotatifin, and hydroxychloroquine. In particular, the last one is the golden standard and it is employed in several clinical protocols.[2−5] Other details regarding its anticovid-19 activity are expected from ongoing clinical trials although some side effects have already been reported. In fact, hydroxychloroquine causes side effects at heart, liver, and kidney and in addition, it could worryingly decrease glucose concentration in the blood.

Surprisingly, the results of the mentioned paper reported that PB28, a cyclohexylpiperazine derivative, designed, synthesized, and largely studied in our laboratories, was 20 times more potent than hydroxychloroquine in anti Covid-19 activity in the viral titer assay, while it displays low affinity for the hERG ion channel, with possibly less cardiac side effects than hydroxychloroquine.[1]

About 20 years ago, our group studied arylpiperazine derivatives as serotonergic (5-HT1A) and dopaminergic (D2-like receptors) ligands for obtaining a novel class of antipsycothic drugs devoid of extrapyramidal effects. Structure activity relationship studies led us to verify that the switch from aryl piperazine to alkyl or cycloalkyl piperazine moiety led to a dramatic reduction of the activity toward serotonergic and dopaminergic receptors, whereas the affinity toward sigma-1 and sigma-2 receptors was in the nanomolar range.[6−9] These preliminary results encouraged us to develop potent and selective sigma receptor ligands, and PB28 (laboratory label) emerged for its subnanomolar[10] affinity and potent sigma-1 antagonist/sigma-2 agonist activity.[11] Considering that these receptors are largely expressed in several organs and in particular in cancer tissues, PB28 has been evaluated as anticancer agent in several tumor cell lines (breast, prostate, glioma neuroblastoma) giving interesting results in this field.[12,13] In order to characterize PB28 interacting proteins, we chemically linked PB28 to a stationary phase column. Crude proteins from lysed human SK-N-SH neuroblastoma cells (where sigma-2 receptor subtypes are overexpressed) were eluted and several proteins were identified by MALDI-MS and LC-MS analysis supported by Mascot MS-MS ion search program. The proteins were identified as human histone proteins:

H3.3A histone (NCBI: 51859376), H2B histone (NCBI: 1568557), H2A.5 histone (NCBI: 70686), H1 (NCBI:

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22770677), and H2.1 histone (SwissProt accession number: P16403).

These results led us to formulate two hypotheses: sigma-2 receptors could be histones or PB28 binds histone proteins besides sigma receptors. The title of our paper in 2006 was: "Is the sigma2 receptor a histone binding protein?"[14] In a competitive binding assay performed on the reconstituted H2A/H2B dimer with [^{3}H]PB28 as radioligand, unlabeled PB28 displayed IC_{50} = 0.50 nM.[15,16] In addition, [^{3}H]PB28 was found to accumulate with up to a 5-fold excess in nuclear fractions over cytosolic fractions of SK-N-SH and MCF7 cells, indicating that PB28 is capable of entering the nucleus to interact with histone proteins. Molecular modeling of the H2A/H2B dimer with PB28 docked in it suggested the possible key interactions of the ligand with the histone proteins. Nevertheless, subsequent confocal microscopy studies did not show nuclear accumulation/entrance of PB28 fluorescent derivatives, keeping the issue of the PB28 interaction with histones unsolved.[17,18]

In the same period, we evaluated the sigma-2 agonist activity of PB28, in single SK-N-SH cells, demonstrating that PB28 abolishes the Ca++ release through the inositol 1,4,5-trisphosphate (InsP3) receptors and ryanodine receptors. In SK-N-SH cells, PB28 incubation for 45 min abolishes the cytosolic Ca++ increases evoked by carbachol or histamine. This effect is due to direct binding at InsP3 receptors localized in high concentration in the endoplasmic reticulum (ER).[19]

Gordon et al. reported that NSP6 is the sigma-involved protein in the anticovid activity.[1] Moreover, Benvenuto et al.[20] demonstrated that NSP6 locates at the ER, where the presence of multiple phenylalanine residues has been recognized, and NSP6 modulates autophagy. It has been shown that this binding could favor coronavirus infection because of the minor ability of autophagosomes to deliver viral components to lysosomes for degradation.

Since sigma-ligands bind ER at InsP3 receptor, PB28 activity toward Covid-19 could be due to its modulation of the NSP6-ER binding. The hydrophobic nature of PB28 (Figure 1) could

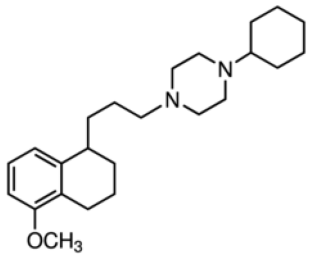

Figure 1. PB28 structure.

drive NSP6 binding via hydrophobic interactions while the lysosomal leakage and oxidative stress induced by PB28 could induce cytoprotective autophagosomes accumulation. These hypotheses could be ascertained for sigma-2 ligand PB28 considering that, as reported by Wileman et al., coronavirus NSP6 proteins restrict autophagosome expansion, formed by omegasomes, from endoplasmic reticulum whether they are due to NSP6 proteins compromising the ability of autophagosomes to deliver viral component to lysosomes degradation.[21,22]

In conclusion, our research group, that has synthesized and biologically characterized PB28 as a subnanomolar sigma receptor ligand (sigma-1 antagonist and sigma-2 agonist), hypothesizes that the more potent effect of PB28 against COVID-19 than hydroxychloroquine, could be due to the potent sigma-2 receptor activity of PB28 that binds InsP3 receptors largely expressed in ER where NSP6 viral protein restricts autophagosome expansion.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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Prof. Roberto Perrone and Prof. Berardi have carried out the research as PI in the first period (1996−2000) leading our research group to meet the results reported in the manuscript.

ABBREVIATIONS

NSP6, nonstructural protein 6 of coronavirus; PB28, 1-cyclohexyl-4-(3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-n-propyl)piperazine

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